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The Effect of Temperature and Enzyme Concentration in the Transesterification Process of Synthetic Microalgae Oil

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Throughout the world, the fossil fuel has supplied around the 80% of the energetic requirements, in Colombia alone 95.1% of energetic demand is made by the transportation sector solely, supplied by oil, kerosene, gasoline and diesel, this sector has an extremely small participation with biofuel of 3%, which is represented only by biodiesel.
Microalgae had been proposed as biofactories with a remarkable third generation biofuels production. The culture of the microorganism comprehends interesting characteristics as countless environments where its natural growth could be replicated in fresh, salty and even sewage waters, with a higher growth rate and a higher oil production. The implementation of enzymes in the transesterification process have generated a good curiosity in the field, due to its mild reactions conditions, lesser energetic requirements, a high standard in the selection of the enzymes with the objective of avoiding the formation of soaps, creating in this way cleaner products and sub-products, in which the separation of the phases biodiesel/glycerol, give the possibility to recuperate the bio catalyzer and high output of reactions. However, the high volume of medium required to obtain lipids is one of the major drawbacks to test the viability of these enzymes. The present study aims to design an enzymatic transesterification process for the production of biodiesel form synthetic *Chlorella* oil. The synthetic oil was designed according to the lipid profile of C 16:0, C16:1, C18:0, C18:1, C18:2 and C18:3 from *Chlorella* spp CHL2 cultured on Bold Basal media under limited concentrations of $NaNO_3$.
The enzymatic transesterification efficiency was evaluated by the implementation of a 2^2 experimental factorial design (temperature and lipase concentration) under a 3: 1 molar ratio of alcohol:oil and a fixed reaction time of 6 hours. The obtained results show that, in order to obtain superior yields of biodiesel (>91%) the transesterification process must be carried out under temperature conditions close to 38°C and lipase concentrations of 5%.

1. Introduction

Fossil fuels have an important role in the coverage of global energy demand (Neumann and Jeison, 2015). In Colombia, 95.1% of this demand is supplied by oil, kerosene, gasoline and diesel (UPME 2009). However, factors such as the decrease in oil reserves, the environmental difficulties caused by its implementation (Garibay *et al.,* 2009), and the rise in oil prices have favored the search for new sustainable sources of energy (Uribe Gomez, 2010). This need has postulated biofuels due to its renewable and biodegradable nature (FEDEBIOCOMBUSTIBLES, 2015). Within the diverse group of raw materials made viable as a replacement for oil, microalgae are presented as a sustainable alternative. The cultivation of these microorganisms can develop in freshwater, saltwater and even in wastewater (Garibay *et al.,* 2009), they also have a higher growth rate, and an oil production by area greater than the oilseed ground crops used for the production of biodiesel (Arias Peñaranda *et a.l*, 2013; Neumann and Jeison, 2015). The microalga *Chlorella* sp has been proposed for its potential for the deposition of oil, with a maximum content of 43% and 52% in dry weight (Arias Peñaranda *et al.,* 2013). Its oil is converted to biodiesel usually by transesterification, where the TAGs react with a short chain alcohol (usually methanol) in the presence of a catalyst to form glycerol and methyl esters of

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fatty acids (FAME's), the latter constitute biodiesel as a biodegradable fuel by not emitting toxic gases (Guschina *et al,* 2013; Murica *et al.,* 2013; Cobos Ruiz *et al.,* 2014); However, in order to increase biofuel efficiency downstream processing still requires further investigation for its successful implementation (Guldhe *et al.,* 2016).

From the catalyst technologies available for biodiesel production, enzyme catalyst has been proved as a greener option due to its reduced energy requierments and high specificity (Guldhe *et al.,* 2017), Lipases are capable of catalyzing both esterification of free fatty acids and transesterification of triglycerides. This gives lipase catalysts an advantage over conventional chemical catalysts while using feedstock lipids containing high free fatty acid (Guldhe *et al.,* 2016). Biodiesel profuction from vegetable oils using enzymatic processes has been extensively studied over the last years (Navarro Lopez *et al.,* 2016).

Microlagae oils can be converted into biodiesel using different types of catalysts such as alkaline or acidic catalysis (depending on the oil profile); however alkali catalyst cannot be used on algae oils due to its high content of free fatty acids which encourage the production of foam (Navarro Lopez *et al.,* 2016), reducing the final efficiency and increasing the downstream cost of biodiesel (Robles-Medina *et al*., 2009), therefore microalgal oils with high FFA contents can be transesterified by enzymatic catalysts. However, Implementation of biocatalyst for algal lipids conversion is novel approach which still requires extensive research (Guldhe *et al.,* 2016). The objective of this work is to evaluate the effect of temperature and enzyme concentration in the production of biodiesel from synthetic oil from *Chlorella* sp.

2. Materials and methods

2.1 Microalgae production

Chlorella sp CHL2 strain from Universidad Francisco de Paula Santander (Colombia) was used (Jaimes *et al*., 2012). The algae were cultured on Bold Basal medium (Andersen *et al.,* 2005) during 30 days in 10 L airlift plastic PBR viously steam sterilized (120°C, 60 min), each experiment was coupled to a bubbling aeration system with an air flow of 0.6 L/L of culture media. In order to increase the amount of lipids, 0,2 g/L of sodium bicarbonate ($NaHCO_3$) were added every 5 days. Biomass was recovered by flocculation with aluminum chloride and lyophilized. Lipids extraction was done according to Bligh & Dyer (Moheimani *et al.,* 2013); finally, the extracted oil was characterized by GC-MS.

2.2 Construction of synthetic microalgae oil

According to the GC-MS results, a synthetic oil profile was constructed using vegetable oils according to eq 1 and 2 reported by Plata *et al* (2009), where ***W*** represents the mass of oil, ***C*** the mass composition of acids fat in oil, ***A*** microalga oil, ***B*** sunflower oil, ***C*** olive oil, ***1*** saturated, ***2*** unsaturated

$$W_B C_{B1} + W_C C_{C1} = W_A C_{A1} \quad (1)$$

$$W_B C_{B2} + W_C C_{C2} = W_A C_{A2} \quad (2)$$

2.3 Transesterification procedure

The effect of the temperature and the concentration of the lipase enzyme XX-25 split (Proenzymes SA) over FAME's production of was determined using a factorial design 2^2 (Table 1), each experiment was performed in triplicate. For each experiment 250 g of synthetic oil were preheated to 110°C for 15 min to remove moisture; enzyme was mixed with the moisture-free oil for 15 minutes, methanol was added at a 3:1 alcohol:oil molar ratio with a reaction time of 6 hours.

After completion of the transesterification reaction the enzyme was inactivated at 110°C for 15 min. the mixture was separated by decantation for 12 hours and the upper phase (biodiesel) was retained and washed 5 times by spraying with water at 55°C with a 2:1 biodiesel:water ratio followed by drying at 110°C for 15 min to remove remaining water. The final product was physicochemically characterized (density, refraction index, corrosion, cinematic viscosity and acidity) and analyzed by gas chromatography with selective mass detector (GC - MS) for the determination of methyl esters of fatty acids (FAME 's) and the biodiesel yield was determined by equation proposed by Moreno (2013). Finally, FAME's were analyzed with the ranges stipulated by EN 14214 and ASTM D 6751.

Table 1: Experimental design

Experiment	Enzyme Concentration (w/w)	Temperature (ºC)	FAME's percentaje (%)
1	5	33	R1
2	5	38	R2
3	10	33	R3
4	10	38	R4

3. Results and discussion

3.1 Construction of synthetic microalgae oil

The fatty acid composition for the microalgae oil was compared to the profile of other vegetable oils (Table 2). Accordingly, olive and sunflower oils due to their similarity in the concentration of monounsaturated fatty acids with the algae oil According with eq 1 and 2, in order to obtain 1 kg of synthetic oil of microalgae it was obtained that 439.33 g of sunflower oil and 565.43 g of olive oil were required.

Table 2: Lipid profile for different oils

Type of fatty acid	Microalgae Oil	Sunflower Oil	Linseed Oil	Olive Oil	Palm Oil
% saturated	14,50	11,20	9,8	16,94	89
% mono-insaturated	82,06	84,75	1,29	79,92	8,9
% di–insaturated	3,42	3,43	N/A	3,01	1,6
% tri-insaturated	N/A	N/A	86,88	N/A	N/A

3.2 Physicochemical analysis of biodiesel

The results for the characterization of each experiment are presented in Table 3. From the results it is important to note that the values of acidity and corrosion are within the values established by the regulations, giving biodiesel obtained good properties such as minor corrosion of engine parts (Herrea and Velez, 2008; Sanchez and Huertas, 2012); on the other hand, the values of density and viscosity are outside the norm. High viscosity levels could cause problems in the injectors and the pump system, shortening the life of the engine, and generating power losses Gamboa and Celis, 2010; Sanchez and Huertas, 2012).

Table 3: Lipid profile for different oils

Parameter	E1	E2	E3	E4	EN 14214	ASTM D6751
Density 15ºC (g/mL)	0,95 ± 0	0,95 ± 0	0,95 ± 0	0,95 ± 0	0,88 – 0,9	0,88 – 0,9
Refraction Index (n^{40} D)	1,48 ± 0	1,47 ± 0	1,48 ± 0	1,47 ± 0	-	-
Acidity (KOH_{mg}/Oil_g)	0,53 ± 0,2	0,65 ± 0,2	0,64 ± 0,1	0,57 ± 0,1	0,5 máx	0,8 max
Cinematic Viscosity (mm^2/g)	13,46 ± 1,0	13,66 ±0,34	12,50 ± 0,4	11,34 ± 0,37	3,5 – 5,0	1,9 – 6,0
Corosion	1a	1a	1a	1a	No.1	No. 3
Yield (%)	87,42 ± 5,8	91,35 ± 1,1	85,49 ± 1,69	89,88 ± 1,875	-	-

Between experiments, E2 (5% w/w of enzyme, 38°C) achieved the best average performance with 91.35%, followed by experiment E4 (89.88%) which was developed under the same temperature but a higher enzyme concentration (10% w/w). These results demonstrate that an increase in enzyme concentration may directly affect the yield of the reaction. However, an increase in temperature under the same concentration of enzyme increases the yield of the reaction by 4%. The results presented in this work are superior to those reported by Calvo & Ladero, (2010); which, when transesterifying olive oil and sunflower with 20% (w/w) of Lipase B *Candida antartica* at 45°C, methanol:oil molar ratio 3:1 for 6 hours obtained yields of 65.18 and 50% respectively. This confirms that a significant increase in the concentration of the enzyme can adversely affect the performance of the process.

3.3 FAME's analysis

FAME's characterization is presented in figure 1, where the presence of four methyl esters is highlighted and methyl oleate (C18:1) and methyl linoleate (C18:2) are the ones with the highest concentration in the four experiments. Similarly, the content of methyl esters of saturated fatty acids is low (between 8.25% and 10.85%), which confer a better performance at low temperatures (Vivas, 2010). According to the European standard UNE-EN 14105, the maximum concentration of Methyl Linolenate (C18:3) (C18:3) is 12% (w/w); however, in the present study the presence of this methylester was not recorded; therefore, the biodiesel obtained has a high oxidative stability, so the possibility of generating deposits in the engine is minimal (Avellaneda, 2010). These results shown that in order to obtain high TAG conversion values, concentrations of 5% of enzyme are sufficient, since increasing by doubling its concentration increases the viscosity of the medium, making contact between the components difficult and affecting the performance of the process (*Rojas et al.,* 2010). Similarly, no significant variations were found when the reaction temperature increased.

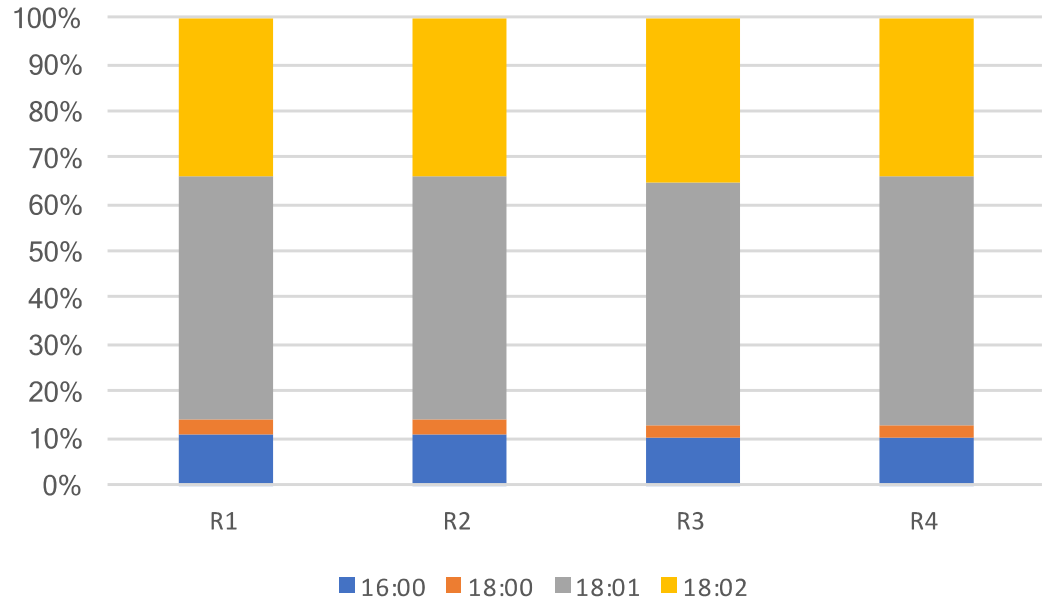

Figure 1. FAME's concentration

4. Conclusions

According to the obtained biodiesel, it was found that the enzymatic transesterification of a synthetic *Chlorella sp* oil is a viable source for the production of biofuels with good characteristics, in which FAME's are present as methyl palmitate (16: 0), methyl stearate (18: 0), methyl oleate (18: 1), and methyl linoleate (18: 2), presenting a greater proportion of monounsaturated fatty acids such as methyl oleate (43.6-57.25%), and with only 14.1% of saturated fatty acids. The above provides biodiesel with adequate lubrication properties and good behavior at low temperatures, as well as a good oxidation stability by not having tri-unsaturated fatty acids. Similarly, the results allow to determine that the temperature does not affect the concentration of FAME's, while the enzyme concentration does affect the performance of the process

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